Spectrum of BAP1 mutations identified in diverse cancer lineages

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Abstract

Background
Germline mutations in the tumor suppressor gene, BAP1, a deubiquitylase that regulates key cellular pathways, are associated with a recently-described tumor predisposition syndrome characterized by early onset benign melanocytic skin tumors, and a significant risk of cancers that include mesothelioma, cutaneous and uveal melanoma, renal cell carcinoma (RCC) and cholangiocarcinoma. Somatic or germline BAP1 mutations have been associated with an aggressive course and a poor prognosis in RCC and cholangiocarcinoma and may render the cancer cells more sensitive to HDAC inhibitors or Parp inhibitors. We investigated types and frequencies of BAP1 mutations in a large cohort of diverse malignancies and their associations with other molecular/genomic characteristics.

Methods:
A total of 9782 tumor samples from over 40 cancer types were molecularly profiled at Caris Life Sciences by next generation sequencing (Illumina NextSeq platform and Agilent SureSelect XT panel, 592 genes). Microsatellite instability (MSI) was tested by PCR and fragment analysis (Promega MSI Analysis System).

Results:
A pathogenic somatic or germline BAP1 mutation was identified in 20 cancer types, with a total of 129 tumors with mutations found (1.3% prevalence). As expected, BAP1 mutations were frequently seen in uveal melanoma (50% or 24 in 48), malignant pleural mesothelioma (29% or 6 in 21), RCC (8% or 12 in 150), cholangiocarcinoma (6.6% or 13 in 196), and cutaneous melanoma (2.2% or 7 in 319). In addition, pathogenic BAP1 mutations were detected in carcinomas arising in parotid gland (10.3% or 3 in 29), anus (8.3% or 3 in 36), cervix (3.4% or 4 in 117), stomach (3.3%, or 6 in 180), head and neck (1 in 97 or 1%), lung (14 in 1590 or 0.9%), and breast (0.8% or 7 in 887). A mutation was also noted in a meningioma (1 in 43) and a uterine sarcoma (1 in 96). Variants of BAP1 (pathogenic, presumed pathogenic, and variants of unknown significance) were more often seen in MSI-high tumors (compared to MSS) in both colorectal (12/61 vs. 18/1003) and endometrial carcinomas (5 of 69 vs. 5 of 321; both $p<0.01$). It was not determined whether the BAP1 mutations were somatic or germline in origin.

Conclusions:
The study confirmed the presence of pathogenic BAP1 mutations in carcinomas commonly associated with BAP1 germline and somatic mutations. It also identified BAP1 mutations in additional cancer types (parotid gland, anal and cervical carcinomas), as well as its association with MSI-H cancers (colon and endometrium). Evaluation of mutations in non-cancerous tissues is underway to determine if these novel cancer associations are related to germline predisposition.

Introduction

BAP1 (BRCA1 associated protein 1) is a deubiquitinase required for efficient assembly of the homologous recombination proteins (BRCA1 and RAD51) at ionizing radiation-induced foci, and promotes error-free repair of these lesions (1). BAP1 tumor predisposition syndrome (BAP1-TPDS) is caused by a heterozygous germline pathogenic variant in *BAP1* gene. It is associated with an increased risk for atypical Spitz nevi and (in descending order of frequency): uveal (eye) melanoma (UM), malignant mesothelioma (MMe), cutaneous melanoma (CM), clear cell renal cell carcinoma (ccRCC), and basal cell carcinoma (BCC). Other suspected but yet unconfirmed tumors in BAP1-TPDS include: breast cancer, cholangiocarcinoma, non-small cell lung adenocarcinoma (NSCLC), meningioma, and neuroendocrine carcinoma (2-4).

Introduction (Continued)

The diagnosis of BAP1-TPDS is established in a proband by identification of a heterozygous germline pathogenic variant in BAP1 on molecular genetic testing. During the molecular profiling of cancers for the purpose of predictive, theranostic interrogations using massively parallel gene sequencing (NGS, Caris Molecular Intelligence, Caris Life Sciences, Phoenix, AZ) we encountered a moderate number of cases with diverse somatic mutations in BAP1 gene. We further evaluated those cases for BAP1 protein expression, microsatellite instability (MSI) and BAP1 gene status in adjacent non-malignant tissues.
The study was approved by the Institutional Review Board (WIRB).

Methods (updated):

Patients and samples:
A total of 14,837 formalin fixed paraffin embedded (FFPE) tumor samples of 49 cancer types were tested in a CLIA/CAP/ISO certified laboratory (Caris Life Sciences, Phoenix, Arizona).

Next Generation Sequencing:
Direct sequence analysis was performed on genomic DNA isolated from a formalin-fixed paraffin-embedded tumor sample using the Illumina NextSeq platform. An Agilent custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets.

Immunohistochemistry:
Staining of the FFPE tissue sections was performed using BAP1 mouse monoclonal antibody (sc-28383 antibody from Santa Cruz Biotechnology) and automated procedures (Ventana Medical Systems, Tucson, AZ). Nuclear staining at any intensity (≥1+) was considered normal expression; lack of nuclear staining (0) was considered abnormal.

Results (Updated)

	Type of Alterations				
Cancer Types	Frame-shift	Intronic Splice Site	Nonsense	Missense	Total
All types	**86**	**47**	**61**	**3**	**197**
Uveal Melanoma	15	13	6	0	**34**
Cholangiocarcinoma	9	5	6	0	**20**
Lung Non-small cell lung cancer (NSCLC)	5	3	11	0	**19**
Kidney Cancer	9	6	2	0	**17**
Occult Primary	5	2	6	2	**15**
Breast Carcinoma	7	2	3	0	**12**
Other	6	2	4	0	**12**
Melanoma	5	2	3	0	**10**
Gastric Adenocarcinoma	6	0	2	0	**8**
Malignant Pleural Mesothelioma	2	2	3	0	**7**
Cervical cancer	2	1	3	0	**6**
Colorectal Adenocarcinoma	1	3	2	0	**6**
Ovarian Surface Epithelial Carcinomas	4	0	0	1	**5**
Uterine Neoplasms - Endometrial carcinoma	1	2	2	0	**5**
Pancreatic Adenocarcinoma	2	1	1	0	**4**
Anal Cancer	0	2	1	0	**3**
Liver Hepatocellular Carcinoma	1	1	1	0	**3**
Extrahepatic Bile Duct Adenocarcinoma	1	0	1	0	**2**
Non-melanoma skin cancer	0	0	2	0	**2**
Esophageal and Esophagogastric Junction Carcinoma	1	0	0	0	**1**
Head and neck Squamous Carcinoma	1	0	0	0	**1**
Meningioma	1	0	0	0	**1**
Prostatic Adenocarcinoma	0	0	1	0	**1**
Small Intestinal Malignancies	1	0	0	0	**1**
Uterine Neoplasms - Uterine sarcoma	0	0	1	0	**1**
Vulvar Cancer (squamous cell carcinoma)	1	0	0	0	**1**

Table 1: Number of BAP1 mutations seen. A total of 197 pathogenic mutations were seen in the 14,837 tumors interrogated. Most frequent alterations seen are frame-shift and nonsense mutations, followed by intronic mutations that disrupt conserved splice sites.

Results (Continued)

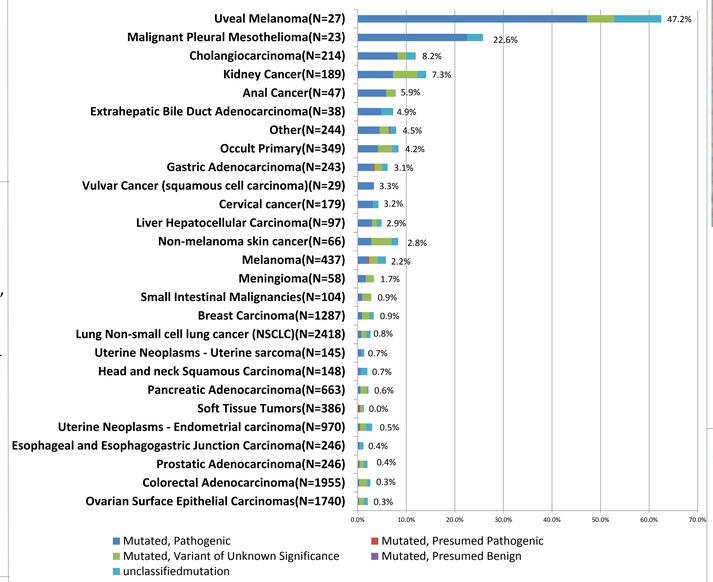

Figure 1. A total of 14,837 tumors of 49 cancer types were tested with NextGen (NextSEQ) and the top 27 cancer types of the highest BAP1 mutation frequency are shown. N numbers of each cancer type tested are shown in the parentheses. Percentages next to the bars represent the frequency of pathogenic mutations .

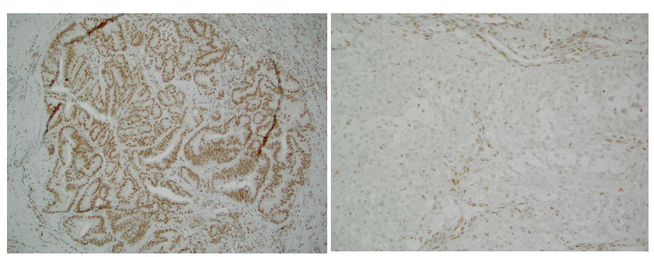

Figure 2: A – retained expression of BAP1 in endometrial adenocarcinoma (*G470R*, 90% a.f.) in a germ line carrier (*G470R* 50% allele frequency in normal tissues). B – Loss of expression of BAP1 in RCC (*BAP1A648fs*) is an example of pathogenic mutation with the loss of protein expression.

Results (Continued)

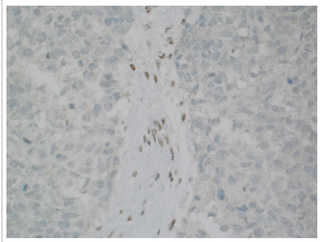

Figure 3. Loss of BAP1 (V599fs) expression in breast carcinoma (normal stromal fibroblasts are positive)

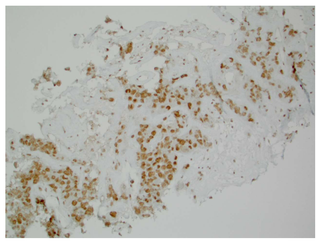

Figure 4. Retained expression of BAP1 in cancer of unknown primary (CUP). Patient with germline BAP1(D608G 50% a. f.) VUS.

Conclusions

1. Interrogation of a large cohort (N=14,837) of solid tumors from a variety of cancers (49 organs/cancer types) reveals BAP1 mutations in 26 different cancer types.
2. In addition to known BAP1-associated cancers (including uveal and cutaneous melanoma, malignant pleural mesothelioma, kidney cancer and cholangiocarcinoma), pathogenic BAP1 mutations were also seen in 6% of anal carcinoma, 4% of cancer of unknown primary and 3% of gastric cancer, vulvar cancer, cervical and liver cancer.
3. Most frequently observed mutations of BAP1 are frame-shifts, nonsense mutations and intronic mutations that alter splice sites; missense mutations are rare.
4. Loss of protein expression is seen to associate with frameshift mutations while retained protein expression is seen in germline BAP1 point mutation cases.
5. Evaluation of mutations in non-cancerous tissues is underway to determine if these novel cancer associations are related to germline predisposition.

References

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